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The keratin 16 null phenotype is modestly impacted by genetic strain background in mice

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Abstract

The type I intermediate filament keratin 16 (K16) is constitutively expressed in ectoderm-derived appendages and is inducibly expressed in the epidermis upon barrier-compromising challenges. Dominantly-acting missense alleles in *KRT16* are causative for pachyonychia congenita (PC), a genodermatosis involving debilitating palmoplantar keratoderma (PPK), nail dystrophy, oral lesions and, frequently, alterations in glands and hair. *C57Bl/6;Krt16*$^{-/-}$ mice develop oral lesions early after birth and PC-like PPK lesions as young adults. These PPK lesions have a marked dysregulation of skin barrier related genes and innate immunity effectors (e.g., danger-associated molecular patterns), and are preceded by oxidative stress secondary to hypoactive Nrf2 signaling. These molecular features are present in PPK lesions of PC patients. Here we report that all components of the *C57Bl/6;Krt16*$^{-/-}$ mouse phenotype occur as well in the *FVB* strain background, albeit less severely so, a significant observation in light of variations in the clinical presentation of individuals harboring disease-causing mutations in the *KRT16* gene.

Introduction

Keratin 16 (K16), a type I intermediate filament gene and protein, is constitutively expressed in specific cell types in ectoderm-derived epithelial appendages (e.g., hair follicles, nail, glands, oral papillae etc.) and is inducibly expressed when stratified epithelia such as the epidermis are experiencing stress (e.g., UV exposure, wounding) or disease (e.g. psoriasis, carcinoma) (1). Genetic linkage analyses and DNA sequencing determined that dominantly-acting missense alleles in *KRT16* are causative for pachyonychia congenita (PC) (2), a genodermatosis involving painful and debilitating palmoplantar keratoderma (PPK), nail dystrophy, oral lesions and, frequently, alterations in glands and hair as well (3). Focal non-epidermolytic PPK lesions, which occur at pressure points in palms and soles, involve dramatic epidermal thickening and hyperkeratosis and are often signficantly painful for patients. Of note, similar alleles in *KRT16* can elicit a presentation of focal non-epidermolytic PPK in the absence of other lesions characteristic of PC (4), suggesting that the consequences associated with alterations in *KRT16* are subject to modifier gene(s) effects.

Mice null for *Krt16*, originally generated in the *C57Bl/6* background, develop oral lesions early after birth and PC-like PPK lesions as young adults (5). Follow-up studies showed that the lesions in footpad skin involve a gross misregulation of skin-barrier related genes, including Danger-Associated Molecular Patterns (DAMPs; also known as alarmins) and effectors of innate immunity (6), and are preceded and caused by a state of oxidative stress secondary to misregulation of Keap1-Nrf2 signaling (7). Several of the key features of PPK-like lesions in footpad skin of *C57Bl/6*;$Krt16^{-/-}$ mice are present in PPK lesions of individuals with PC (6,7). Here, we describe the phenotype associated with the *Krt16* null allele in the *FVB* mouse strain background. This study was conducted for the primary purpose of defining the role of *Krt16* in HPV16-induced carcinogenesis in the *FVB* background (see [8]) but provided an opportunity to assess the impact of genetic strain background on expression of the $Krt16^{-/-}$ phenotype.

Results and Discussion

The *Krt16* null allele was transferred to the *FVB* strain background via six sequential backcrosses of *C57Bl/6;*$Krt16^{+/-}$ and wildtype *FVB* mice. Intercrosses between *FVB;*$Krt16^{+/-}$ were next conducted and the progeny analyzed. A third of *FVB;*$Krt16^{-/-}$ offspring dies within a day after birth, while ~50% of these mice survive to adulthood (Fig 1A), which is slightly more than what had been observed in the *C57Bl/6* strain (by 17%; [5]). Macroscopic examination at postnatal day 5 (P5) reveals whitish and raised plaques (oral leukoplakia) in the posterior aspect of the dorsal tongue epithelium *FVB;*$Krt16^{-/-}$ mice, but not in *FVB;*$Krt16^{+/-}$ or *FVB;*$Krt16^{+/+}$ mice (Fig 1B). Histologically, examination of sagittal cross sections of this area of the dorsal tongue reveals a mildly thickened, disorganized and occasionally cytolytic epithelium (Fig. 1C), with a normal complement of oral papilla (data not shown), at P5. Immunostaining of similar tissue sections reveals that expression of the differentiation-related K13 (Fig. 1D) and its partner K4 (data not shown) are unaltered, whereas that of K17 is dramatically reduced in the dorsal tongue epithelium of *FVB;Krt16*$^{-/-}$ mice (Fig. 1E). Altogether these observations, summarized in Suppl. Fig. 1, show that the survival at weaning and the character of oral lesions in *FVB;Krt16*$^{-/-}$ mice are similar to, but not as severe, as those previously reported for *C57Bl/6;Krt16*$^{-/-}$ mice (5).

At the two months mark post-birth, surviving *FVB;Krt16*$^{-/-}$ mice still weigh less compared to *WT* littermates (Suppl Table 1). All surviving male and female *FVB;Krt16*$^{-/-}$ mice develop PPK-like lesions on their front paws (Fig. 2A), as we reported for *C57Bl/6;Krt16*$^{-/-}$ mice (5). Male mice exhibit a slightly earlier onset of PPK relative to females (average 4.67 ± 0.37 vs. 5.04 ± 0.41 weeks; see Fig. 2D) and also exhibit a greater PPK index, a macroscopic and blinded measure of lesion severity, at 6 weeks of age (Fig 2, A and E). A similar sex bias for PPK onset also occurs in *C57Bl/6;Krt16*$^{-/-}$ mice (9). Histologically, the paw skin of *FVB;Krt16*$^{-/-}$ mice exhibit thickened epidermis and modest hyperkeratosis starting at four weeks (Fig. 2B) and peaking at eight weeks (Fig. 2C; sex-specific quantitation reported in Fig. 2F). Lesional footpad skin shows decreased staining for filaggrin (Fig 2H), suggesting impaired epidermal differentiation, a ~2.5 fold increase in phosphorylated Histone H3-positive nuclei in the basal layer of *FVB;Krt16*$^{-/-}$ relative to *FVB;Krt16*$^{+/+}$ epidermis, indicating a mild state of hyperproliferation (Fig. 2G), and cells positive for the innate immune cell marker CD11b, suggesting an inflammatory infiltrate (Fig. 2I). These observations are qualitatively similar to those made when characterizing *C57Bl/6;Krt16*$^{-/-}$ mice (5) (see Suppl Fig 1).

Next we assessed some of the key molecular readouts that had been shown to be misregulated in footpad skin of *C57Bl/6;Krt16*$^{-/-}$ mice. Emphasis was placed on mRNA levels for DAMPs (danger-associated molecular patterns; see (6)), key effectors of the glutathione-based antioxidant system, and of the Keap1-Nrf2 system (7). Transcript levels for *Krt6a*, the type II partner gene for *Krt16* and a sensitive indicator of cell and tissue stress, and of several DAMPS including *DefB4*, *S100A8*, and *S100A9* were markedly elevated (Fig. 2J). Additionally, the mRNA transcript levels for *Sprr2d* and *SerpinB3a*, which contribute to genesis of the cornified envelope, were also markedly elevated (Fig. 2J). Some of these findings were corroborated at the protein level, e.g., by western blotting for S100A8 and the activated form of IL-1β (data not shown). These observations suggest that while qualitatively similar, the alterations occurring in the skin barrier are less severe in *FVB;Krt16*$^{-/-}$ relative to *C57Bl/6;Krt16*$^{-/-}$ mice.

One of the key justification of this set of experiments was to assess the impact of *Krt16* loss for HPV16$^{tg/+}$-induced tumorigenesis in *FVB* mouse skin (see (8)). *FVB;Krt16*$^{+/-}$ mice were thus bred to the *FVB;HPV*$^{tg/+}$ mouse line as described (8). Remarkably, 100% of the *Krt16*$^{-/-}$*;HPV*$^{tg/+}$ mice died within two weeks after birth (Suppl Table 2). While the cause of death remains undefined and calls for substantive follow-up studies, the outcome of this cross is indicative of a strong genetic interaction between the HPV16 transgene and the *Krt16* null allele.

In conclusion, our findings suggest that while genetic strain background does not impact the presentation of the *Krt16* null phenotype in mouse at a qualitative level, it does influence the intensity of virtually all aspects of the presentation, ranging from perinatal lethality to the severity of oral and PPK-lesions. Differences in inflammatory responses between *C57Bl/6* and *FVB* strains may account for these observations (10,11). These findings are thus consistent with the variable penetrance of the clinical presentation in individuals whose genome harbor mutated *KRT16* alleles (2-4), and point to the *Krt16* null mouse model as a useful resource to define the determinants involved.

Materials and Methods

The methods used in this study are described under “Supplemental Materials”.

Acknowledgments

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Conflict of Interest

The authors report no conflict of interest.

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Legends to Figures

Figure 1. *FVB;Krt16$^{-/-}$* mice have increased mortality compared to WT and develop oral lesions. (A) Survival curve of *FVB;Krt16* mice. n=31-36 mice/genotype. ** denotes p<0.01 determined by Log-rank test. (B) Pictures of macroscopic tongue plaques that occur in *FVB;Krt16$^{-/-}$* mice (both sexes) at P5. (C) H&E staining of P5 tongues to evaluate histology of female *FVB;Krt16$^{-/-}$* tongue lesions. Scale bar, 50 µm (D and E) Immunostaining for K13 and K17 in sections of fresh frozen tongue tissue from P5 male littermates.

Figure 2. *FVB;Krt16$^{-/-}$* mice develop PPK lesions. (A) Images of 6-weeks old male (right) and female (left) *FVB;Krt16$^{-/-}$* mice displaying macroscopic appearance of PPK lesions compared to controls. (B and C) H&E staining of front paw skin sections relating histological changes in 4- and 8-weeks old *FVB;Krt16$^{-/-}$* male mice. Dotted line corresponds to dermal/epidermal junction. Epi, epidermis; SC, stratum corneum. Scale bar, 50 µm. (D) Quantitation of PPK lesion onset in both sexes of *FVB;Krt16$^{-/-}$* mice. Mice were scored as lesional when evidence of macroscopic lesions (waxy keratoderma) were present. n=4-5 mice/sex. (E) Quantitation of PPK lesions severity based on presence of waxy keratoderma on front paws of 6-week old *FVB;Krt16* mice. n=2-4 mice/sex/genotype. (F) Sex-specific quantitation of epidermal thickness from 8-week old *FVB;Krt16* front paws. n=2-3 mice/genotype. (G) Quantitation of proliferation (measured by P-Histone H3 staining) in front paw skin from 8-weeks old male *FVB;Krt16* mice. n=3 mice/genotype. (H) Immunostaining for filaggrin (green) and keratin 5 (red) in front paw skin from 8-weeks old male *FVB;Krt16* mice. (I) Immunostaining for CD11b (green) and p65 (red) in front paw skin from 8-weeks old male *FVB;Krt16* mice. Scale bar, 50 µm. (J) Transcript levels for Danger Associated Molecular Patterns (DAMPs) in front paw skin from 8-week old *FVB;Krt16* null mice relative to *WT* controls. * denotes p<0.05 and ** denotes p<0.01 as determined by unpaired 2-tailed Student's *t* test on dCq values.

Figure 1 - Zieman & Coulombe

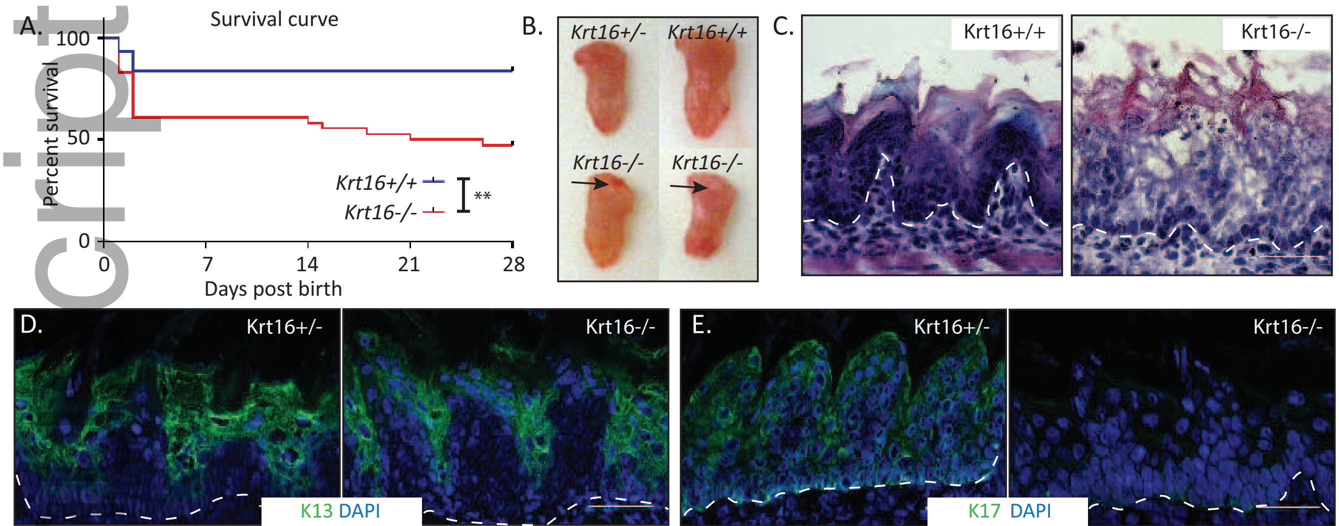

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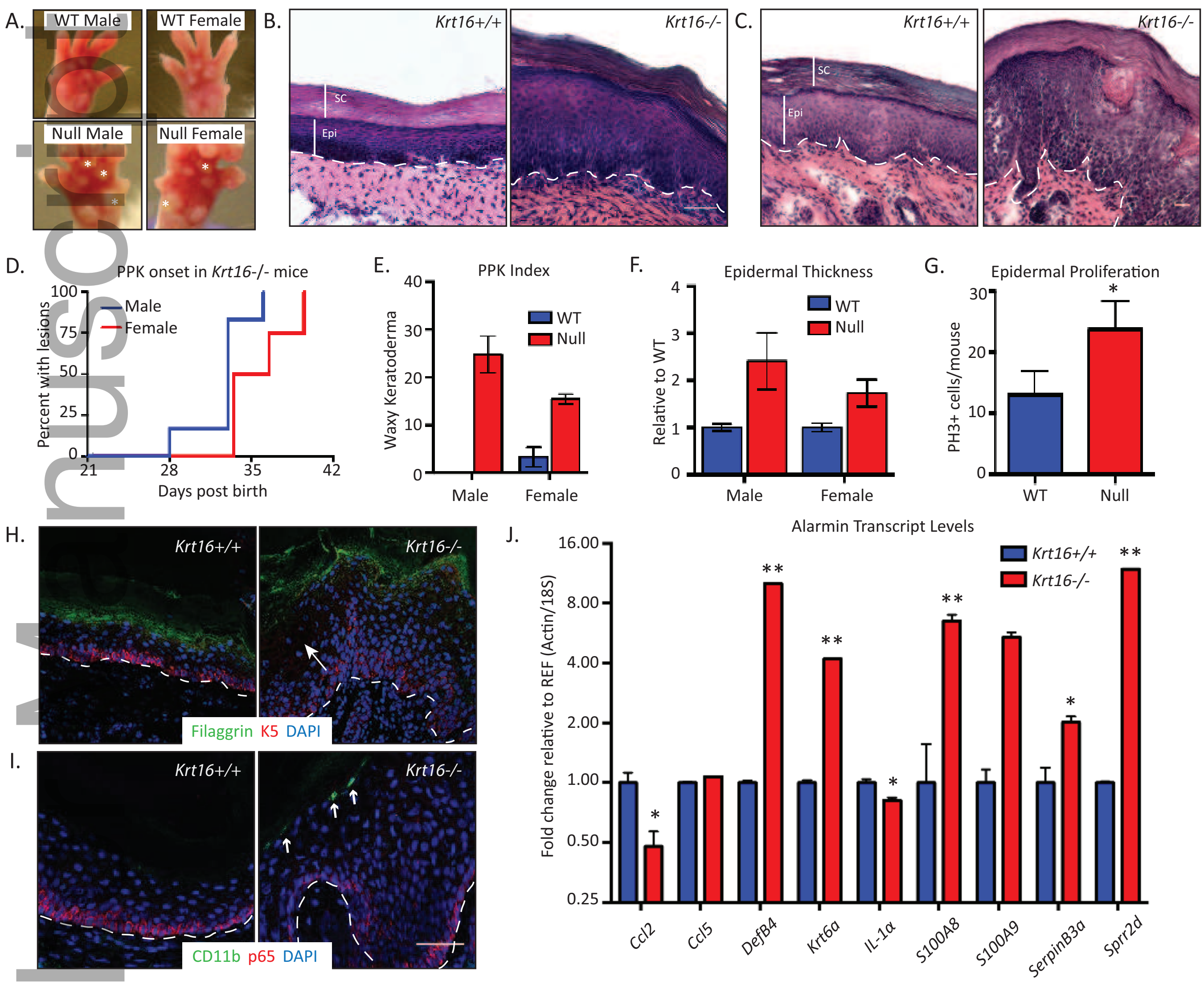
Figure 2 - Zieman & Coulombe



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